SDS – POLYACRYLAMIDE GEL OF OSTEOINDUCTIVELY ACTIVE PROTEINS FROM HPLC

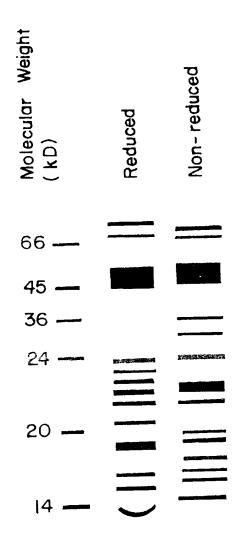
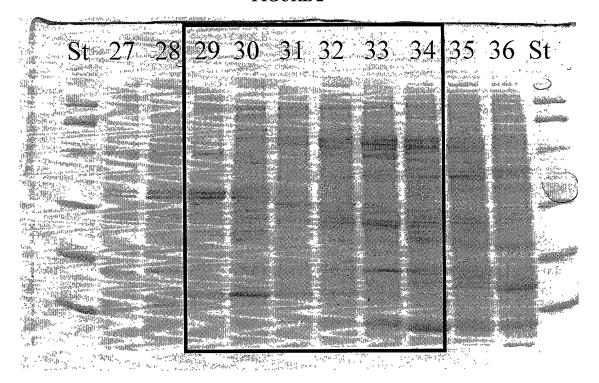


FIG. 1

FIGURE 2



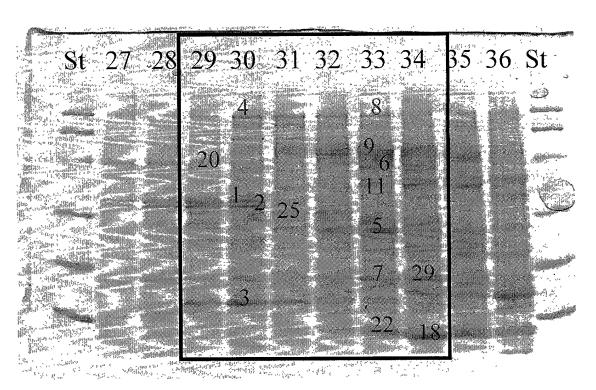
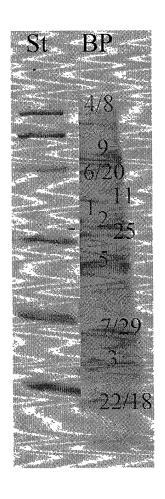


FIGURE 3



Band No.	Identity
1	Histone H1.c
2	Histone H1.c
3	Ribosomal protein RS20
4	Similar to ribosomal protein LORP
5	BMP-3
6	α2 macroglobulin RAP and BMP-3
7	Similar to ribosomal protein LORP
8	BMP-3
9	BMP-3
11	Ribosomal protein RL6 and BMP-3
18	TGF-β2 / SPP 24
20	Factor H
22	TGF-β2
25	BMP-3 and H1.x
29	BMP-3 and ribosomal protein RL32

FIGURE 4

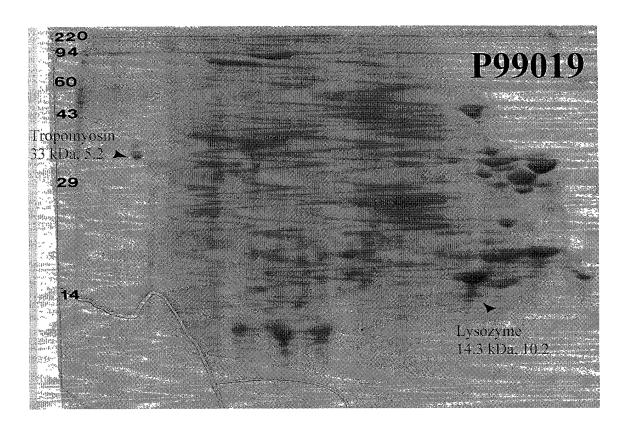


FIGURE 5

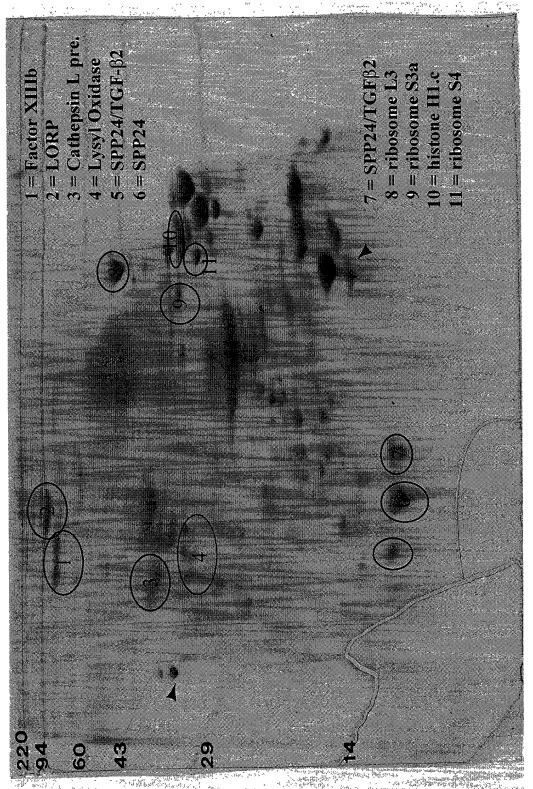


FIGURE 6

Figure 7A (Band 1)

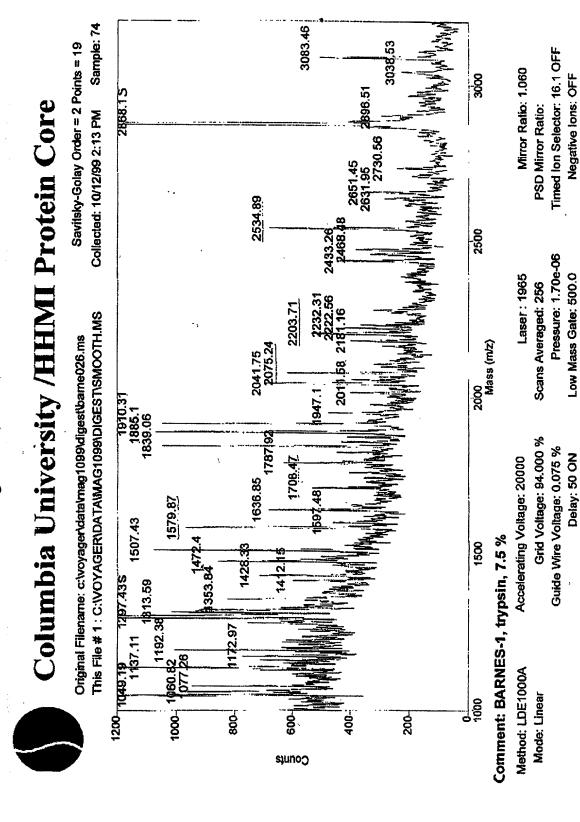
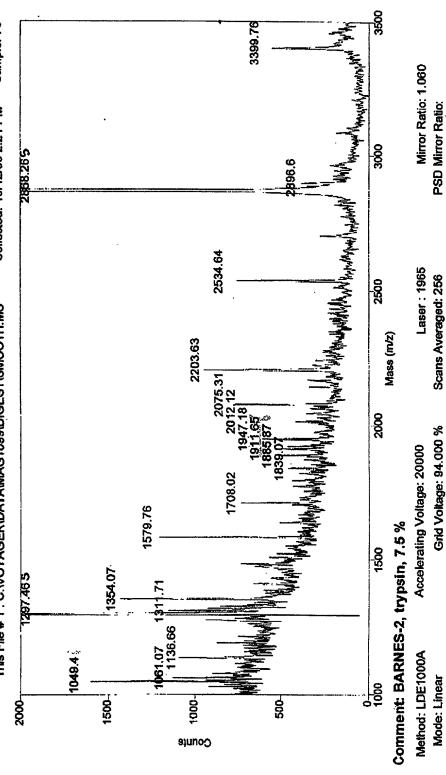


Figure 7B (Band 2)



Original Filename: c:\voyager\data\mag1099\diges\thearthame027.ms Savits
This File # 1 : C:\VOYAGER\DATA\MAG1099\DIGEST\SMOOTH.MS Collected

Savitsky-Golay Order = 2 Points = 19 Collected: 10/12/89 2:21 PM Sample: 75



Timed fon Selector, 16.1 OFF

Pressure: 1.58e-06

Guide Wire Voltage: 0.075 %

Delay: 50 ON

Low Mass Gate: 500.0

Negative Ions: OFF

Figure 7C (Band 3)

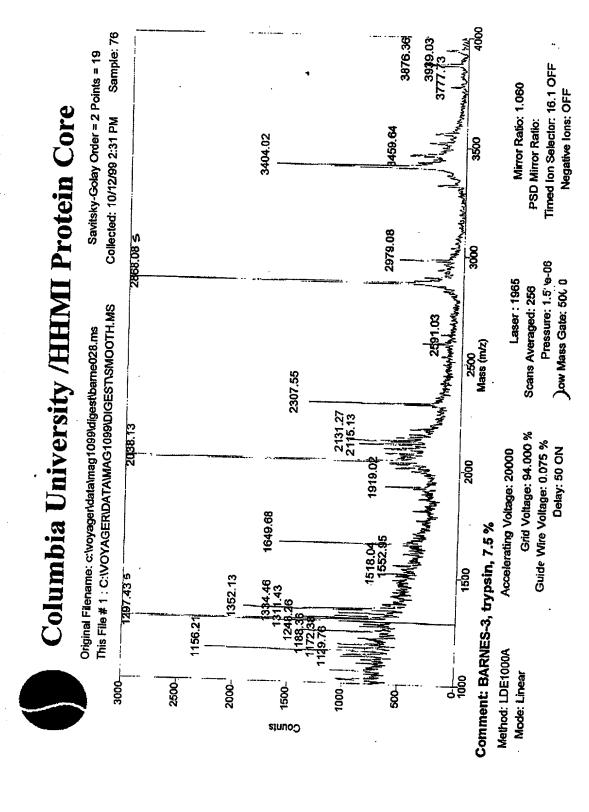
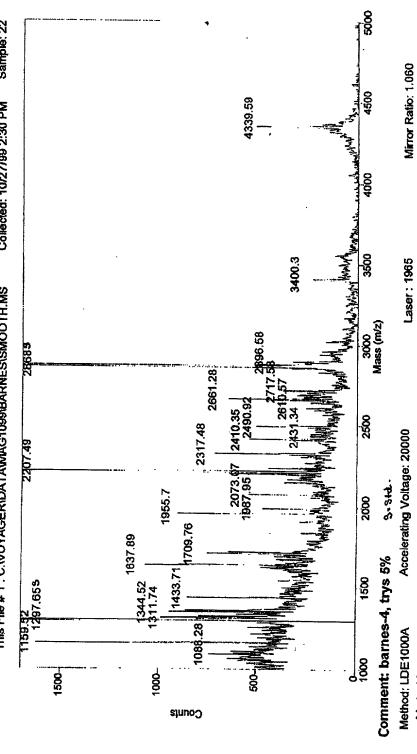


Figure 7D (Band 4)





Savitsky-Golay Order = 2 Points = 19 Collected: 10/27/99 2:30 PM Sample: 22



Timed Ion Selector: 16.1 OFF

Pressure: 9.04e-07

Scans Averaged: 256

Grid Voltage: 94,000 %

Mode: Linear

Guide Wire Voltage: 0.075 %

Delay: 50 ON

Low Mass Gate: 500.0

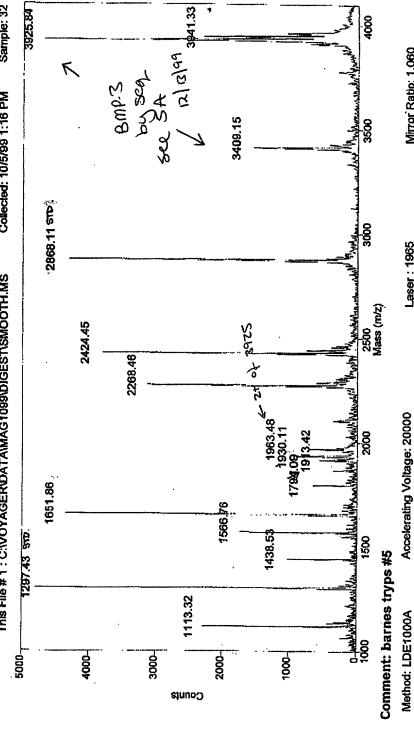
PSD Mirror Ratio:

Negative lons: OFF

Figure 7E (Band 5)

This File # 1: C:\VOYAGER\DATA\MAG1099\DIGEST\SMOOTH.MS Original Filename: c:\voyager\data\mag1099\diges\bare002.ms

Sample: 32 Savitsky-Golay Order = 2 Points = 19 Collected: 10/5/99 1:16 PM



Timed Ion Selector: 16.1 OFF

Pressure: 3.68e-07

Scans Averaged: 121

Grid Voltage: 94.000 %

Mode: Linear

Accelerating Voltage: 20000

Guide Wire Voltage: 0.075 %

Delay: 50 ON

Low Mass Gate: 500.0

Laser: 1965

PSD Mirror Ratio:

Negative lons: OFF

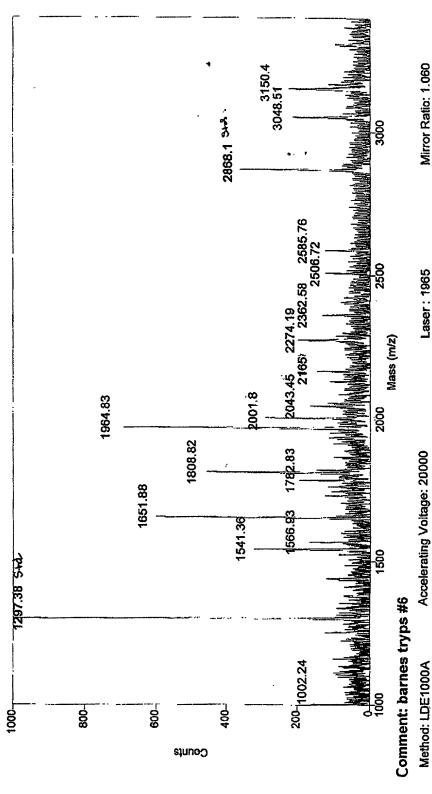
Mirror Ratio: 1.060

Figure 7F (Band 6)

Protein Core Columbia University /HHM

Original Filename: c:\voyager\data\mag1099\digest\barne001.ms This File # 1 : C:\VOYAGER\DATA\MAG1099\DIGEST\SMOOTH.MS

Savitsky-Golay Order = 2 Points = 19 Collected: 10/5/99 1:14 PM Sample: 33



Timed Ion Selector: 16.1 OFF

Pressure: 4.06e-07

Scans Averaged: 256

Grid Voltage: 94.000 %

Mode: Linear

Guide Wire Voltage: 0.075 %

Delay: 50 ON

I nur Mace Cater 500 0

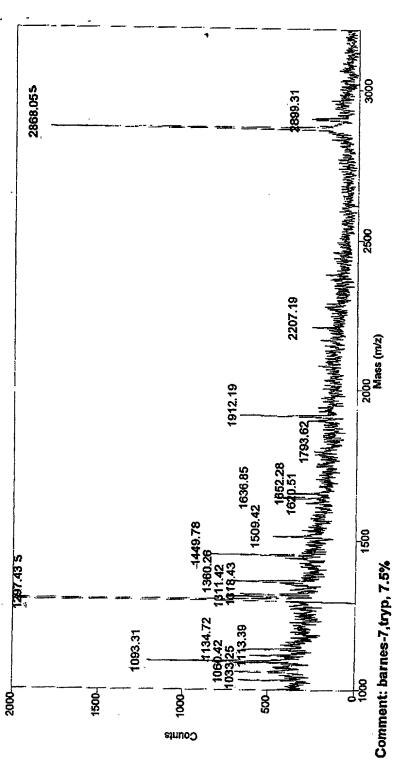
PSD Mirror Ratio:

Figure 7G (Band 7)



Original Filename: c:\voyager\data\mag1199\diges\tsnow_004.ms Strict # 1 : C:\voyager\data\mag1199\diges\tsnow_00\text{GEST}

Savitsky-Golay Order = 2 Points = 19 Collected: 11/10/99 3:11 PM Sample: 65



Timed Ion Selector: 16 1 OFF

Pressure: 5.89e-07

Scans Averaged: 256

Grid Voltage: 94,000 %

Accelerating Voltage: 20000

Method: LDE1000A Mode: Linear Guide Wire Voltage: 0.075 %

Delay: 50 ON

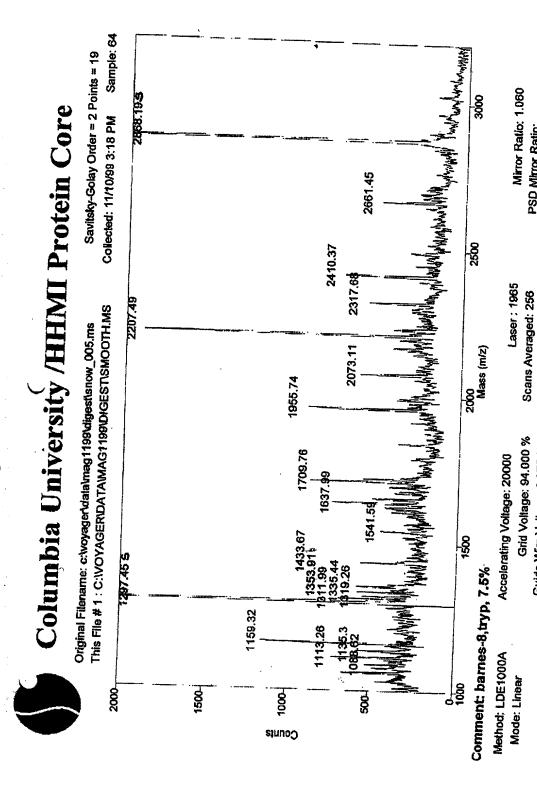
_ow Mass Gate: 500.0

PSD Mirror Ratio:

Negative Ions: O-

Mirror Ratio: 1.060

Figure 7H (Band 8)



Timed Ion Selector: 16.1 OFF

Pressure: 4.01e-07

Guide Wire Voltage: 0.075 %

Delay: 50 ON

Low Mass Gate: 500.0

PSD Mirror Ratio:

Negative Ions: OFF

Figure 7I (Band 9)

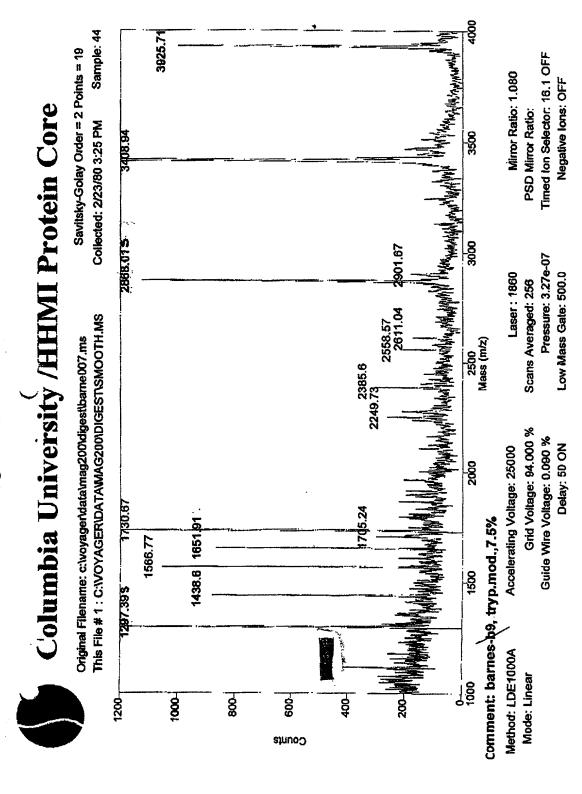
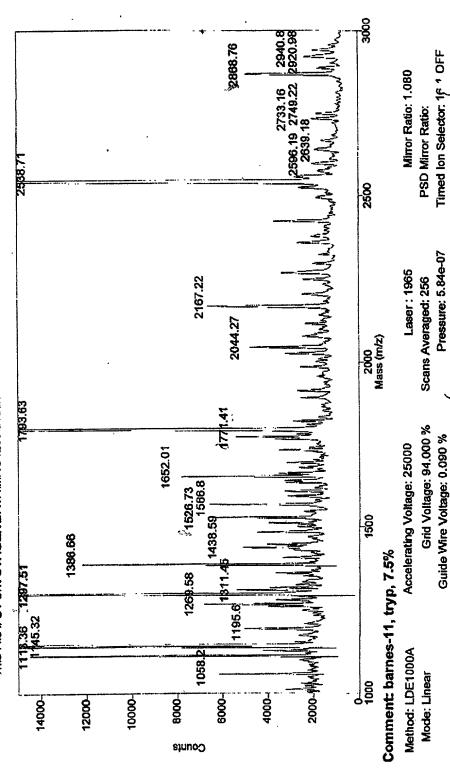


Figure 7J (Band 11)



Original Filename: c:\voyager\data\mag1299\digest\bane004.ms This File # 2 : C:\VOYAGER\DATA\MAG1299\DIGEST\SMOOTH.MS

Savitsky-Golay Order = 2 Points = 19 Collected: 12/15/99 3:49 PM Sample: 14



Negative lons: ८, .

...ow Mass Gate: 500.0

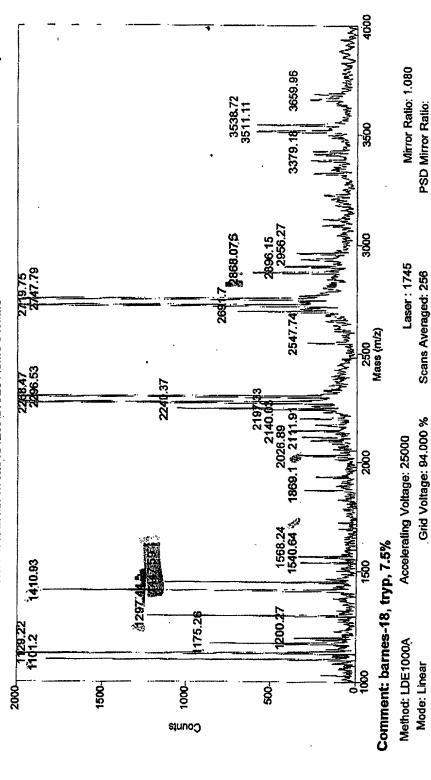
Delay: 50 ON

Figure 7K (Band 18)



Original Filename: c:\voyager\data\rmag1299\digest\barne005.ms This File # 2 : C:\VOYAGER\DATA\MAG1289\DIGEST\SMOOTH.MS

Savitsky-Golay Order = 2 Points = 19 Collected: 12/15/99 4:47 PM Sample: 13



Timed Ion Selector, 16.1 OFF

Pressure: 2.90e-07

Guide Wire Voltage: 0.090 %

Delay: 50 ON

Low Mass Gate: 500.0

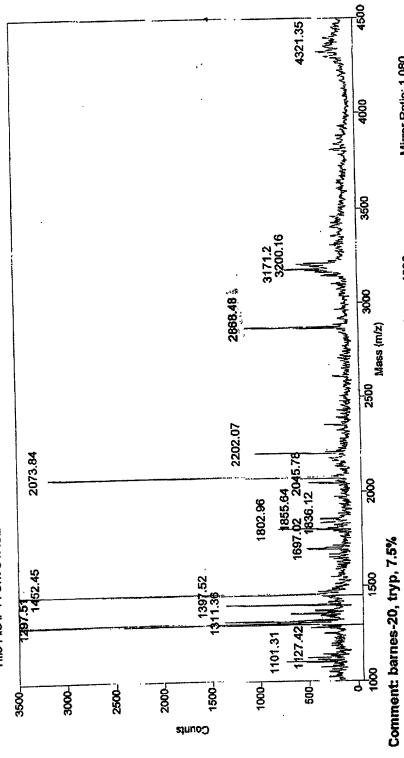
Negative lons: OFF

Figure 7L (Band 20)

This File # 4 : C:\VOYAGER\DATA\MAG100\DIGEST\SMOOTH.MS Original Filename: c:\voyager\data\mag100\digest\bane001.ms

Savitsky-Golay Order = 2 Points = 19 Collected: 1/6/80 3:36 PM

Sample: 45



Timed Ion Selector: 16 * OFF

Pressure: 9.21e-07

Scans Averaged: 256

Grid Voltage: 94.000 %

Accelerating Voltage: 25000

Method: LDE1000A Mode: Linear

Guide Wire Voltage: 0.090 %

Delay: 50 ON

Laser: 1820

Low Mass Gate: 500.0

PSD Mirror Ratio:

Negative lons: Or-

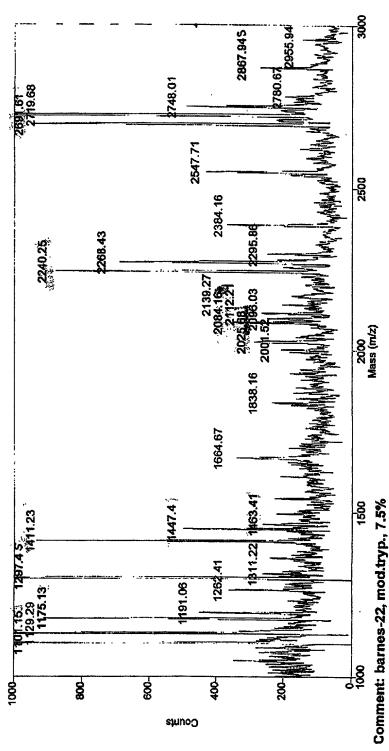
Mirror Ratio: 1.080

Figure 7M (Band 22)

Protein Core Columbia University /HHMI

This File # 1: C:WOYAGERIDATAIMAG200DIGESTISMOOTH.MS Original Filename: c:\voyager\data\mag200\digest\barne003.ms

Sample: 54 Savitsky-Golay Order = 2 Points = 19 Collected: 2/16/80 3:35 PM



Method: LDE1000A Mode: Linear

Grid Voltage: 94.000 % Accelerating Voltage: 25000

Guide Wire Voltage: 0.090 % Delay: 50 ON

Mirror Ratio: 1.080 PSD Mirror Ratio:

Timed Ion Selector: 16 1 OFF

Pressure: 3.43e-07

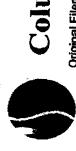
Scans Averaged: 231

Laser: 1870

...ow Mass Gate: 500.0

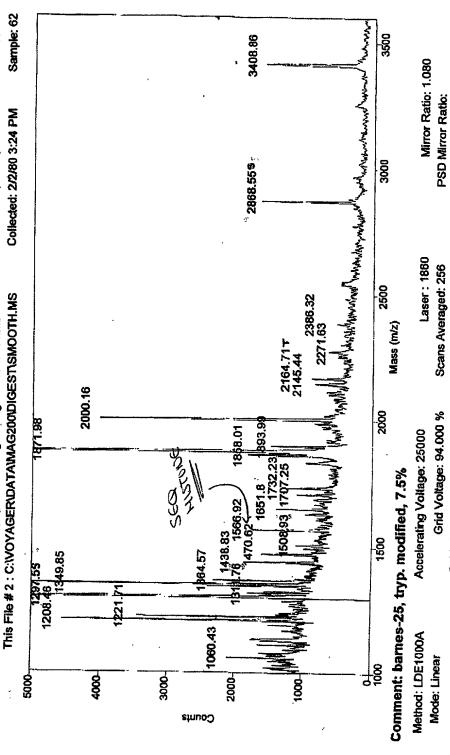
Negative lons: ひ

Figure 7N (Band 25)



Original Filename: c:\voyager\data\mag200\diges\bame001.ms

Savitsky-Golay Order = 2 Points = 19



Timed Ion Selector: 16 'OFF

Pressure: 9.26e-07

Guide Wire Voltage: 0.090 %

Delay: 50 ON

LOW Mass Gate: 500.0

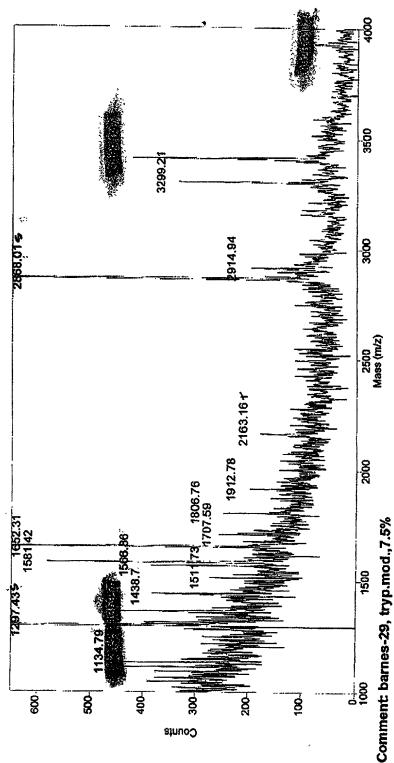
Negative Ions: Or-

Figure 70 (Band 29)

This File # 2: C:\VOYAGER\DATA\MAG200\DIGEST\SMOOTH.MS Original Filename: c:\voyager\data\mag200\digest\barne006.ms

Savitsky-Golay Order = 2 Points = 19 Collected: 2/23/80 3:19 PM

Sample: 43



Timed Ion Selector: 16.1 OFF

Pressure: 3.82e-07

Scans Averaged: 256

Grid Voltage: 94.000 %

Accelerating Voltage: 25000

Method: LDE1000A Mode: Linear Guide Wire Voltage: 0.090 %

Delay: 50 ON

Laser: 1860

Low Mass Gate: 500.0

Negative lons: OFF

Mirror Ratio: 1.080

PSD Mirror Ratio:

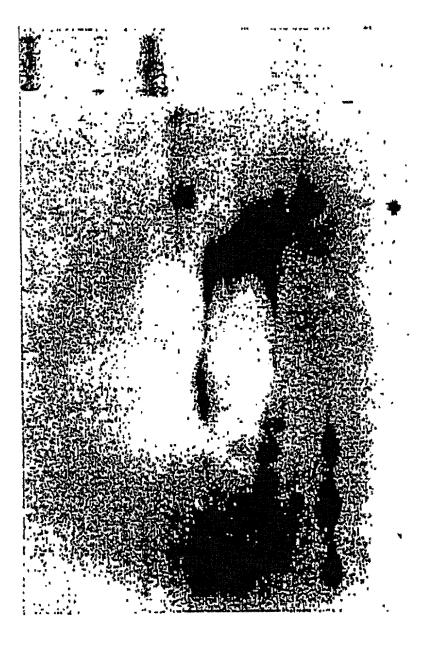


Figure 8

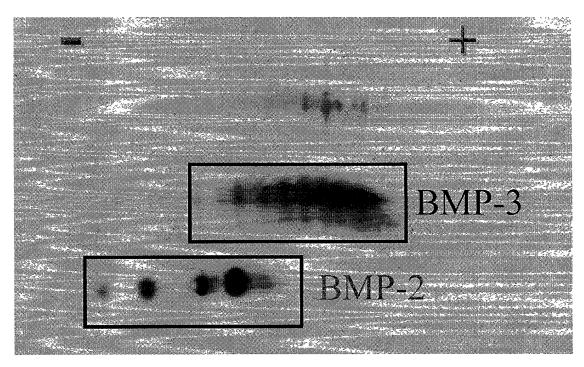


FIGURE 9A

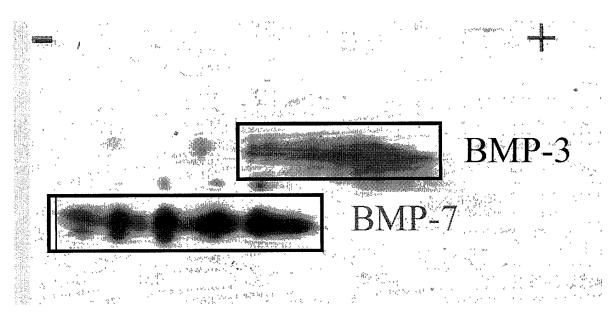


FIGURE 9B

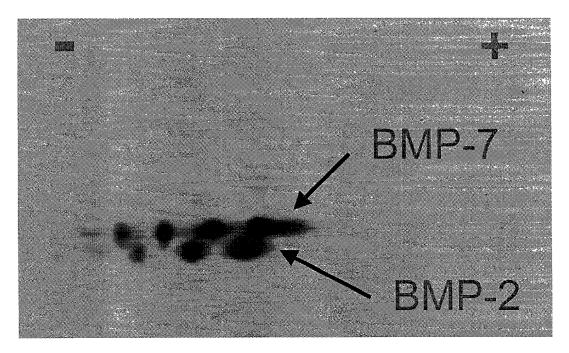


FIGURE 9C

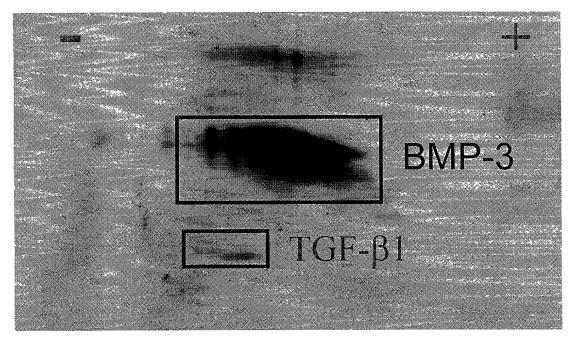
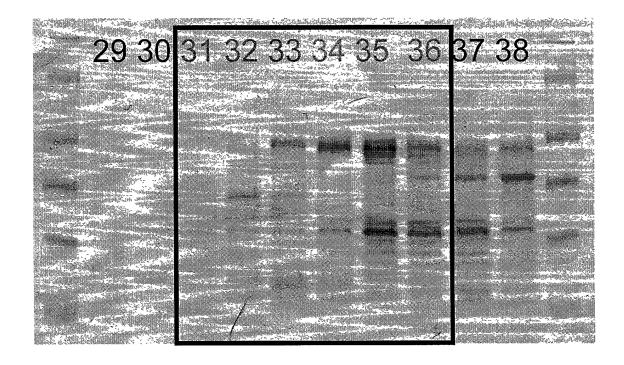


FIGURE 9D

FIGURE 10







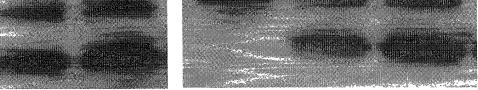
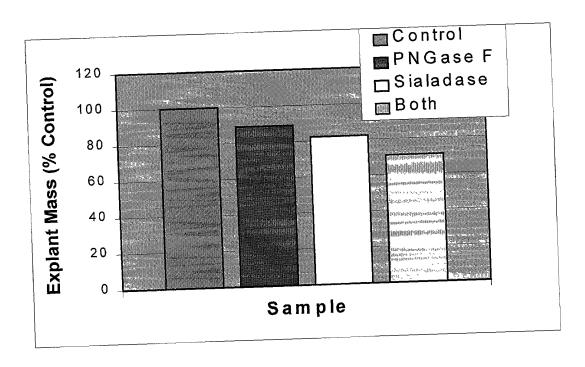


FIGURE 11 FIGURE 12

FIGURE 13A



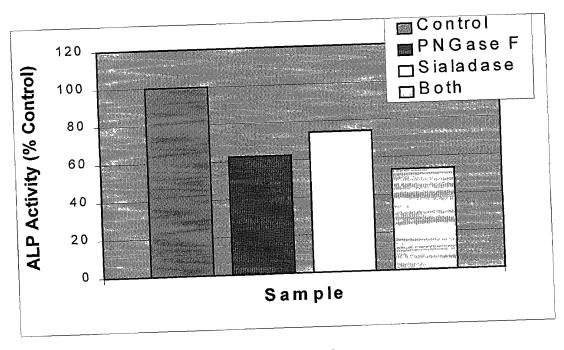


FIGURE 13B

FIGURE 14: Antibody Listing

Specificity	Antigen	Host Species	PC/MC	Source	Catalog No.	
TGF-β1 (human)	Protein	Rabbit	Polyclonal	Promega	G1221	
TGF-β2 (human)	Peptide	Rabbit	Polyclonal	Santa Cruz Biotechnology	sc-90	
TGF-β3 (human)	Peptide	Rabbit	Polyclonal	Santa Cruz Biotechnology	sc-82	
BMP-2 (human)	Protein	Rabbit	Polyclonal	Austral Biologics	PA-513-9	
BMP-3 (human)	Peptide	Chicken	Polyclonal	Research Genetics	NA	
BMP-4 (human)	Peptide	Goat	Polyclonal	Santa Cruz Biotechnology	so-6896	
BMP-5 (human)	Peptide	Goat	Polyclonal	Santa Cruz Biotechnology	sc-7405	
BMP-6 (human)	Peptide	Mouse	Monoclonal	Novocastra Laboratories	NCL-BMP6	
BMP-7 (human)	Peptide	Rabbit	Polyclonal	Research Genetics	NA	
FGF-1 (human)	Peptide	Goat	Polyclonal	Santa Cruz Biotechnology	sc-1884	
osteonectin (bovine)	Protein	Mouse	Monoclonal	DSHB	AON-1	
osteocalcin (bovine)	Protein	Rabbit	Polyclonal	Accurate Chemicals	A761/R1H	
serum albumin (bovine)	Protein	Rabbit	Polyclonal	Chemicon International	AB870	
transferrin (human)	Protein	Chicken	Polyclonal	Chemicon International	AB797	
apo-A1 lipoprotein (human)	Protein	Goat	Polyclonal	Chemicon International	AB740	

ndyksozs okosot

Figure 15A: Identification of Proteins by Amino Acid Sequencing of Tryptic Fragments from 1D Gels

Band	Sample	Sequence Data	Best Database Match	Match	Identification	Species	Accession No.	AAs
-								
2	fx 49 (1579)	fy 49 (1579) XI AAAGYDVEK	ALAAAGYDVEK	11/11	histone H1.c	human	87668 (NCBI)	65-75
1 ~	fv 67 (1346)		SLEKVCADLIR	11/11	40s Ribosomal Protein S20	rat	R3RT20 (PIR)	31-41
4		WYCGMLGFPSEAPV	VVCGMLGFPGEKRV	11/14	LORP	mouse	AAC95338 (NCBI)	213-226
· L	N terminal sed		STGVLLPLQNNELPG	15/15	BMP-3	human	4557371 (NCBI)	290-304
,	fv 72 (3925)	AEYOY	STGVLLPLQNNELPGAEYQY	20/20	BMP-3	human	4557371 (NCBI)	290-309
	fx 74 (3409)	STGVII PLO	STGVLLPLQ	6/6	BMP-3	human	4557371 (NCBI)	290-298
ဇ	fx 55 (1566)	(S)QTLQFXE	SQTLQFDE	2/8	BMP-3	human	4557371 (NCBI)	346-353
•	fx 47		no match		222			
 	N terminal sed	N terminal sed HAGKYSREKNT(P)A(P)	HGGKYSREKNQPKP	11/14	α2-Macroglobulin Receptor Assoc. Pro.	human	P30533 (Swiss-Prot)	31-46
	fx 57 (1438)	SOTLOFDEO	SQTLQFDEQ	6/6	BMP-3	human	4557371 (NCBI)	346-354
	fx 57 (1652)		SLKPSNHA	8/8	BMP-3	human	4557371 (NCBI)	410-417
7	fx 51 (1093)	fx 51 (1093) AALRPLVKP	AALRPLVKP	6/6	60s Ribosomal Protein L32	mouse	P17932 (Swiss-Prot)	1-9
	fx 37 (no MS)	fx 37 (no MS) A(H)I(O)VERYV	AIVER	5/5	60s Ribosomal Protein L32	mouse	P17932 (Swiss-Prot)	109-113
	fx 37 (no MS)	fx 37 (no MS) A(H)I(Q)VERYV	HQSDRYV	2/2	60s Ribosomal Protein L32	mouse	P17932 (Swiss-Prot)	22-28
8	fx 78 ()	XALF(G)AQLGXALGPI	no match		297			
6	fx 56 (1567)	fx 56 (1567) SQTLQFDEQT	SQTLQFDEQT	10/10	BMP-3	human	P12645 (Swiss-Prot)	346-355

DTVLEDE DLIGH

Figure 15B: Identification of Proteins by Amino Acid Sequencing of Tryptic Fragments from 1D Gels

Band	Sample	Sequence Data	Best Database Match	Match	Identification	Species	Accession No.	AAs
		100 PT 10						
11	fx 55 (1311) SQTLXF	SQTLXF	SQTLQF	9/9	BMP-3	human 4	4557371 (NCBI)	346-351
	fx 47 (1772)	fx 47 (1772) VLATVTKPVGGDK	VLATVTKPVGGDK	13/13	60s Ribosomal Protein L6	human	uman Q02878 (Swiss-Prot)	87-99
	fx 76 (1795) xVFAL	xVFAL.	VFAL	4/4	60s Ribosomal Protein L6	human	Q02878 (Swiss-Prot)	273-276
-	fx 61 (1145)	fx 61 (1145) AVPQLQGYLR	AIPQLQGYLR	9/10	60s Ribosomal Protein L6	human	numan Q02878 (Swiss-Prot)	262-271
18								
22	fx 58 (1101)	fx 58 (1101) ALDAAYCFR	ALDAAYCFR	6/6	TGF-β2	human		303-311
	fx 69 (no match)	x 69 (no match) GYNANFCAGACPYL	GYNANFCAGACPYL	14/14	TGF-82	human	P08112 (Swiss-Prot)	340-353
	fx 66 (1411.71) VNSQSLSPY	VNSQSLSPY	VNSQSLSPY	6/6	SPP24	bovine	bovine Q27967 (Swiss-Prot)	42-50
25	fx 39 (1470)	fx 39 (1470) KAAKPSV(P)	KAAKPSVP	8/8	Histone H1 x	human	JC4928 (PIR)	199-206
20		14000						

Figure 16A: Identification of Proteins by Mass Spectrometry of Tryptic Fragments from ID Gels

Comments			15 MS peaks match with Band 2		Out of the second secon	identification of starred peptide	confirmed by sequence analysis	15 MS peaks match with Band 1							and the second s	12 MS peaks match with Band 8		
% Coverage	/0 CO C C R 8 C	22				16			62							9		
AAc	Š	110-121	62-29	64-79	35-54	65-79*	64-79	35-54	50-59	76-83	26-66	88-99	9-21	5-21	88-119	150-167	648-669	455-478
Mass	Difference	09.0	0.16	0.58	-0.74	0.05	0.13	-0.20	0.36	60.0-	-0.16	0.55	0.27	-0.17	-0.85	-0.32	-0.28	0.47
Mass Spec	Database	1172.37	1579.71	1707.89	2012.32	1579.71	1707.89	2012.32	1129.40	1156.30	1334.62	1351.58	1517.77	1919.19	3404.87	1988.27	2410.63	2610.10
Mass Spec	Data	1172.97	1579.87	1708.47	2011.58	1579.76	1708.02	2012.12	1129.76	1156.21	1334.46	1352.13	1518.04	1919.02	3404.02	1987.95	2410.35	2610.57
Accession	Number	87668 (NCBI)				87668 (NCBI)		,	R3RT20 (PIR)			1				NP002309	(Swice-Prof)	(2011)
	salpade	human				human			rat							human		
oligon Country of the	Mass spec Prome	4 peaks match with	histone H1 c	0.1101018111		3 peaks match with	histone H1 c		7 peaks match with	ribosomo 200						3 peaks match with	I yey Oxidasa BD	Lyayi Ovidaso ivi
	Band	-	•			2	l		m	,						4	-	

Figure 16B: Identification of Proteins by Mass Spectrometry of Tryptic Fragments from ID Gels

	olihoud coord coord	Choolog	Accession	Mass Spec	Mass Spec	Mass	AAs	% Coverage	Comments
Band	Band Mass spec Prome species	Salpade	Number	Data	Database	Difference			
ıc	O neaks match with	himan	_	1113.32	1113.31	0.01	361-368	84	% coverage calculation is relative
)	ם שמם	5		1438.53	1438 58	-0.05	346-357		to the mature BMP-3, 183 AAS
	C-LINIG		(inoni)	1566.76	1566.76	00:00	345-357		(290-472)
			-	1651.86	1621.91	-0.05	410-424		
			•	1794.09	1794.02	0.07	346-360		
				2268 46	2268.63	-0.17	374-392		and the state of t
				2424.45	2424.81	-0.36	373-392		Identification of starred peptide
			-	3409.15	3407.77	1.38	290-318*		confirmed by sequence analysis
9	3 peaks match with human	human	P30533	1002.24	1002.15	60.0	283-290	17	
	α2-Macroglobulin		(Swiss-Prot)	2362.58	2362.43	0.15	129-150		
	RAP			3048.51	3048.52	-0.01	257-282		
	2 peaks match with	human	4557371	1566.93	1566.75	0.18	346-357	15	% coverage calculation is relative
	BMP-3			1651.88	1651.91	-0.03	410-424		to the mature BMP-3, 183 AAS
) 								(290-472)

DOZUBOWE NUMBER

Figure 16C: Identification of Proteins by Mass Spectrometry of Tryptic Fragments from ID Gels

			Accession	Mass Spec	Mass Spec	Mass	800	% Coverage	Comments
Band	Band Mass Spec Profile Species	Species	Number	Data	Database	Difference	e C), core	
7	A peaks match with	molise	P17932	1033.25	1033.17	0.08	67-75	33	
-	ibosomo 199)	/Swice_Brot/	1093.31	1093.40	-0.09	1-10*		
	Ilbosoffie L3Z		(3001) 1-661MC)	1134.72	1134.28	0.44	65-74		
				1449.78	1449.66	0.12	19-29		177
	5 peaks match with	himan	4557371	1060.42	1060.20	0.22	102-111	21	% coverage calculation is relative to
	0 0000		(aCN)	1113.39	1113.31	80.0	361-368		the mature BMP-3, 183 AAS (290-
	S-TIME		(Idoni)	1360.26	1360.58	-0.32	190-200		472)
				1652.28	1651.91	0.37	410-424		
				1793.62	1794 02	-0.40	346-360		
8	1 peak matches with	human	NP002309	2410.37	2410.63	-0.26	648-669	ဧ	12 MS peaks match with Band 4
	Lysyl Oxidase RP		(Swiss-Prot)						
σ	G neaks match with	himan	4557371	1113.14	1113.31	-0.17	361-368	36	% coverage calculation is relative to
·		5	(NCB)	1438.60	1438.58	0.02	346-357		the mature BMP-3, 183 AAS (290-
	DIVIP-0		(100)	1566.77	1566.76	0.01	345-357		472)
				1651.91	1651.61	0 30	410-424		
				2901.67	2901.19	0.48	41-66		
				3408.94	3407.77	1.17	290-318		

Figure 16D: Identification of Proteins by Mass Spectrometry of Tryptic Fragments from ID Gels

Band Mass Spec Profile Species Accession Mass Spec Profile Mass Spec Profile Accession Mass Spec Profile Accession Data adaptases Data adaptases Accession Accession Data adaptases Accession Accessi					- 1		10.00			
Pumber Data Database Difference human 4557371 1113.23 1113.31 -0.08 361.368 48 human 4557371 1651.73 1651.91 -0.18 410.424 48 1 (NCBI) 1793.58 1794.02 -0.44 346-360 346-360 2424.24 2424.81 -0.57 373-392 16 340-360 3408.34 3407.77 0.57 290-318 16 144-122 16 human QO2878 1440.38 1440.23 0.01 262-271 16 mouse P47911 145.68 0.06 260-271 52 fwiss-Prot) 1386.74 1386.88 0.06 260-271 52 human P08112 1101.20 1101.26 -0.06 303-311 52 csyliss-Prot) 2240.37 2240.60 -0.23 312-328 30 csyliss-Prot) 1447.69 0.06 142-53 30 csyliss-Prot) 1447.66 <th> </th> <th>i i</th> <th></th> <th>Accession</th> <th></th> <th>Mass Spec</th> <th>SSS</th> <th>AAs</th> <th>% Coverage</th> <th>Comments</th>		i i		Accession		Mass Spec	SSS	AAs	% Coverage	Comments
human 4567371 1113.23 1113.31 -0.08 361-368 48 (NCBI) 1651.73 1651.91 -0.18 410-424 48 (NCBI) 1793.58 1794.02 -0.44 346-360 410-424 2424.24 2424.81 -0.57 373-392 16 3408.34 3407.77 0.57 290-318 16 Ruman Q02878 1140.38 1140.23 0.15 144-152 16 mouse PA7911 1059.15 1059.12 0.02 141-152 16 mouse PA7911 1145.36 1165.12 0.03 10-20 16 mouse PA7911 1145.36 1165.12 0.01 262-271 16 (Swiss-Prot) 1386.74 1386.88 0.06 260-271 52 bovine Q27967 1477.52 1477.65 0.01 42-53 30 CSwiss-Prot) 1540.69 0.06 142-53 0.06 42-53 30 </th <th>and</th> <th>Mass Spec Profile</th> <th>Species</th> <th>Number</th> <th></th> <th>Database</th> <th>Difference</th> <th></th> <th></th> <th>11.00</th>	and	Mass Spec Profile	Species	Number		Database	Difference			11.00
5 peaks match with bovine Module with bovine	,	dfirst datase select	200	1557371	1113.23	1113.31	80 0-	361-368	48	% coverage calculation is relative to
BMP-3 (NCD) 1793.58 1794.02 -0.44 346-380 5 peaks match with human Q02878 1140.23 2424.81 -0.57 373-392 5 peaks match with human Q02878 1140.38 1140.23 0.65 141-122 16 4 peaks match with human P08112 1059.15 1069.12 0.01 262-271 52 5 peaks match with human P08112 1175.26 1175.42 -0.16 400-409 52 5 peaks match with bovine Q27967 1410.93 1411.60 -0.03 312-328 30 5 peaks match with bovine Q27967 1410.93 1411.60 -0.06 303-311 312-328 5 peaks match with bovine Q27967 1410.93 1447.65 -0.06 13-124 SPP24 SSP24 1869.10 -0.06 62-77 -0.16 400-409 2684.7 2688.57 -0.10 33-63 -0.10 33-53	=	5 peaks match with	5	1975	1651 73	1651.91	-0.18	410-424		the mature BMP-3, 183 AAS (290-
5 peaks match with runan Lough and continuous and contin		BMP-3		(IACDI)	1793.58	1794.02	-0.44	346-360		472)
5 peaks match with ribosome L6 human QO2878 (Swiss-Prot) 1140.38 (1440.23) 1140.23 (0.05) 0.057 (144-122) 4 peaks match with SPP24 human (Swiss-Prot) 1526.88 (1445.36) 1626.88 (0.06) 1626.71 5 peaks match with Povine SPP24 human (Swiss-Prot) 1775.26 (175.26) 1775.42 (0.01) 4447.65 (0.02) 5 peaks match with Povine SPP24 Q27967 (Swiss-Prot) 1447.59 (1447.65 (0.02) 1447.65 (0.02) 1369.16 (0.05) 5 peaks match with Povine SPP24 Q27967 (Swiss-Prot) 1440.60 (0.04) 1640.60 (0.04) 86-98 (0.05) 5 peaks match with SPP24 Q27967 (Swiss-Prot) 1447.65 (0.05) (0.05) 62-77 (0.05) (0.05) 62-77 (0.05) (0.05) (0.05)					2424.24	2424.81	-0.57	373-392		
5 peaks match with human QO2878 (Swiss-Prot) 1140.38 (1526.86 (Swiss-Prot)) 1140.38 (Swiss-Prot) 1140.38 (Swiss-Prot) 1140.38 (Swiss-Prot) 1145.68 (Swiss-Prot) 1145.68 (Swiss-Prot) 1145.36 (Swiss-Prot) 1145.36 (Swiss-Prot) 1145.36 (Swiss-Prot) 1145.35 (Swiss-Prot) 1145.35 (Swiss-Prot) 1101.26 (Swiss-Prot) 1101.26 (Swiss-Prot) 1175.26 (Swiss-Prot) 1175.26 (Swiss-Prot) 1175.26 (Swiss-Prot) 1175.26 (Swiss-Prot) 1141.60 (Swiss-Prot) 1144.63 (Swiss-Prot)					3408.34	3407.77	75.0	290-318		
ribosome L6 (Swiss-Prot) 1526.88 1526.86 0.02 141-155 4 peaks match with Speaks matc		5 peaks match with	human	Q02878	1140.38	1140.23	0 15	114-122	16	
A peaks match with Sporie house bovine P47911 PA911 1059.15 PA7912 1059.12 PA7912 0.03 PA7911 10-20 4 peaks match with Sporie (Swiss-Prot) PO8112 1145.36 PA7911 1145.36 PA7911 100.01 PA9211 100.01 PA9211 100.01 PA9211 100.01 PA9211 100.01 PA9211 100.01 PA9212 100.01 PA9212 <t< td=""><td></td><td>ribosome L6</td><td></td><td>(Swiss-Prot)</td><td>1526.88</td><td>1526.86</td><td>0.02</td><td>141-155</td><td></td><td></td></t<>		ribosome L6		(Swiss-Prot)	1526.88	1526.86	0.02	141-155		
4 peaks match with human P08112 1101.26 1145.35 0.01 262-271 4 peaks match with bovine Q27967 1410.93 1447.65 0.06 138-31 5 peaks match with bovine Q27967 1410.93 1447.65 0.06 113-124 200.00 10.04 86-98 1540.64 1540.64 0.05 62-77 1889.10 1889.05 0.05 62-77 2268.57 0.01 33-53			00100	DA7011	1059.15	1059.12		10-20		
4 peaks match with human P08112 1101.20 1101.26 -0.06 303-311 1075.26 1175.42 -0.16 400-409 2240.37 2240.60 -0.23 312-328 2891.70 2691.91 -0.21 340-362 2891.70 2691.91 -0.06 113-124 SPP24 (Swiss-Prot) 1889.10 1889.05 0.05 62-77 2268.47 2268.57 -0.10 33-53			Denoil	4 th C	1145.36	1145.35		262-271		
4 peaks match with human P08112 1101.20 1101.26 -0.06 303-311 TGF-B2 TGF-B2 (Swiss-Prot) 2240.37 2240.60 -0.23 312-328 5 peaks match with bovine Q27967 1410.93 1447.65 -0.06 113-124 SPP24 (Swiss-Prot) 1869.10 1869.05 62-77 2268.47 2268.57 -0.10 33-53				(SWISS-F10t)	1386.74	1386.68		260-271		
4 peaks litated with 100 bovine Spe24 (Swiss-Prot) 1175.26 1175.42 -0.16 400-409 12240.37 2240.60 -0.23 312-328 2240.37 2240.60 -0.21 340-362 2691.70 2691.91 -0.21 340-362 42-53 1411.60 -0.67 42-53 1417.69 1447.69 113-124 86-98 1540.64 1540.64 1540.60 0.04 86-98 2268.47 2268.57 -0.10 33-53	0	dim dotom min	200	PO8112	1101.20	1101.26		303-311	52	
bovine Q27967 1410.93 1447.69 -0.02 312-328 Swiss-Prot) 1540.64 1540.64 1689.05 0.06 113-124 1869.10 1869.10 1869.05 0.06 62-77	<u>∞</u>	4 peaks match with	2	(C) (100 C)	1175.26	1175.42		400-409		
bovine Q27967 1410.93 1411.60 -0.67 42-53 (Swiss-Prot) 1447.59 1447.65 -0.06 113-124 1540.64 1540.64 1540.60 0.04 86-98 2268.47 2268.47 2268.57 -0.10 33-53		TGF- J 32		(SWISS-FIOR)	2240.37	2240.60		312-328		
bovine Q27967 1410.93 1411.60 -0.67 42-53 (Swiss-Prot) 1447.59 1447.65 -0.06 113-124 1540.64 1540.64 1540.60 62-77 1869.10 1869.10 33-53					2691.70	2691.91	-0.21	340-362		
(Swiss-Prot) 1447.59 1447.65 -0.06 1540.64 1540.60 0.04 1869.10 1869.05 0.05 2268.47 2268.57 -0.10		4000		027967	1410.93	1411.60		42-53	30	
(SWISS-FLOU) 1540.64 1540.60 0.04 1869.10 1869.05 0.05 2268.47 2268.57 -0.10		5 peaks match with		(C. 1100)	1447.59	1447.65				
1869.05 0.05 2268.57 -0 10		SPP24		(SWISS-PIOU)	1540.64	1540.60		86-98		
2268.57 -0 10					1869.10	1869.05				
					2268.47	2268.57	-0 10			

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Figure 16E: Identification of Proteins by Mass Spectrometry of Tryptic Fragments from ID Gels

Band Mass Spec Profile 22 5 peaks match with TGF-β2 2 peaks match with	sec Profile		///	000		23.55	•		- opmoneo
		Species	Number	Mass spec	Database	Difference	AAs	% Coverage	Comments
	dtiver dotom	To Car	P08112	1101.15	1101 26	-0.11	303-311	63	
1 c	Hatch With	5	(Chaice Droft)	1175.13	1175.42	-0.29	400-409		
2 peaks	1GF-J32		(SWISS-FIDI)	2084.16	2084.42	-0.26	312-347		
2 peaks				2240 25	2240.60	-0.35	312-328		
2 peaks				2691.61	2691.91	-0.30	340-362		
	2 peaks match with	bovine	Q27967	1411.23	1411.60	-0.37	42-53		
<i>σ</i> .	SPP24		(Swiss-Prot)	1447.40	1447.65	-0.25	113-124		
+	dtish with	himan	himan IC4928 (PIR)	1208.46	1208.40	90.0	48-57	4	
20 Deaks	o peaks match with			1221.71	1222.35	-0.64	107-118		
histo	nistone H1.x			1349.85	1350.52	29.0-	107-119		
				1364.57	1364.59	-0.02	48-58		
				1732.23	1732.97	-0.74	43-57		
2/000	E poole moteh with	nemind	4557371	1060.43	1060.20	0.23	102-111	34	% coverage calculation is relative to
o peaks	Hatch will	5	(aCN)	1438.83	1438.58	0.25	346-357		the mature BMP-3,183 AAS (280-472)
<u>n</u>	BINIP-3		(Idoni)	1566.92	1566.76	0.16	345-357		
				1651 80	1651 91	-0.11	410-424		
				3408.86	3407.77	1.09	290-318		

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Figure 16F: Identification of Proteins by Mass Spectrometry of Tryptic Fragments from ID Gels

7	Alban Canada	Choolog	Accession	Mass Spec	Mass Spec	Mass	944	% Coverage	Commente
Dalla	Darid Mass obec Profile Species	sapade	Number	Data	Database	Difference	Š)	3
29	29 4 peaks match with	human	4557371	1113.22	1113.31	-0.09	361-368	27	% coverage calculation is relative to
	BMD-3		(NCRI)	1438.70	1438.58	0.12	346-357		the mature BMP-3,183 AAS (290-472)
)		(1)	1566.86	1566.75	0.11	345-357		
				3409.04	3407.77	1.27	290-318		

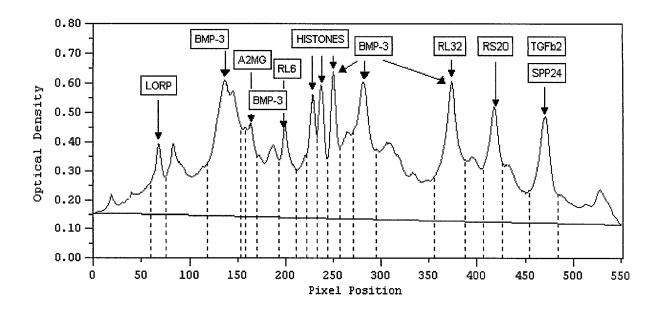


Figure 17A

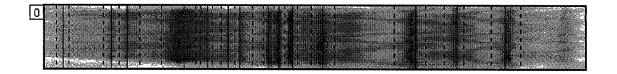


Figure 17B

FIGURE 18: Quantitation of Identified BP proteins

Identified Protein	Percentage of Total Protein
LORP	2
BMP-3	11
BMP-3 and A2-MG	3
RL6 & BMP-3	4
Histone	3
Histone	3
Histone & BMP-3	4
BMP-3	8
RL32 & BMP-3	8
RS2D	5
SPP24 & TGF-β2	6
Total	58%

Figure 19A

Identification of Proteinsby Mass Spectrometry of Fragments from 210 Gels

				_					-	_	•	-	-	Ť	_
Comments					poylide malch confirmed by sequence analysis										
**	Coverage		*		·	2		¥		٠.,		Part 1-1		<u></u>	
AAS			472-4B7	368-382	488-504	-0.51 241-253	848-668	105-116	28-70	21-33	301-314	310-134	774-295	239-261	131-154
		Diff	<u>ь</u>	0.51	YN.	.0.31	0.28	DY'6	0.14	0,36	1.06	0.71	1.40	D. 50	D.43
		Darisbase	1837.14	1921.14	S.	1609,08	2410.63	1606,60	1548.70	1660.80	1680.80	1634.00	2351.50	2380,70	2727.50
PES	Peaks	Date	1857.01	1921.45	2679.51	1609.57	2410.89	14.07.26	1546.BI	(881,16	1881.AS	1834.71	2352.00	2381.50	272(5)
Acc. No.			POS180 (Switz- Profi			NP002308 (SWASS- 1609,57 Proi)		P25075 (Swiss- Prot)	•		•				
Sacries			Herman			Human		Bovine							
Mass Snac			2 peaks maich with Coagulation Factor XIIID			Trypsin 2 peaks match		8 peaks match with Caribepsin I Portursor							•
Spall Dinest			Lyse		,	Trypsin		Lysc							
Strain			-			2	4	6							

Figure 19C

Identification of Proteinsby Mass Spectrumetry of Fragments from 1D Gels

Сопиления												•									
Coverage		42					10			37											
₩		28.35		32-37	BB-107	1-25	42-60			340-355	10-18	286-206	249-250	103-114	103-115	81-18	30-48	151-121	Z	70-90	198-228
	THE L	75.0		-0.25	150-	1.44	0.26			0.25	0.0B	0.22	0.02	71.0	0.04	-0.16	-0.12	0.34	12.0	-0.49	-0.25
	Database	774.80		808.94	1175,43	3166.88	15.1815			917.14	884.15	1192.40	1380.65	1484.63	162D.82	1770.00	2238.55	\$9 52E Z	2581.04	2898.43	2948.35
Postke	Tada	774.56	'	60.608	1175.12	3168.10	2167.77			ec.712	98(23	1192.62	1360.67	1484.60	1620.68	1778.64	7238.43	Z325.99	16,1985	2697.84	2946.10
Ack, Na.		P21214 (Swiss-	FOF				027867 (Swiss-	Pac		P38872 (Swiss- Prof)						•		•	•		
Species		Bordae					Bowine			Bovine											
Mess Spec		4 weaks match	with TGF-62				1 peak	metches with	5	Trypsin 12 peaks match with abosome	3			•					1		
प्रीवस्थ		l ve.C								Trypsin			•							-	
Sport		-								-											

Figure 19D Identification of Proteinchy Mass Spectrometry of Bregments from 2D Gels

ы																					
Comments																					
72	Coverage		02	•						23				23			~				i.
AAs			19-26	152-101	151-161	174-186	94-10B	199-212	65-81	37-16	82-39	61-13		230-239	134-144	182052	198-210	37.48	7	17.98	
		Die	. 8.	0.02	0.13	a DO	-0.10	40.09	-0.04	0.19	10.0	.0.24	-0.38	a,50	00'0	. OAZ	0.12	-023	•	410	
	-	Datalase	DZD, 10	1218.31	1348.49	1516.69	1593.82	1720.00	1053,16	1327.58	157075	4707 Rg	2147.53	1 (66.38	(2/6,39		15D7.0B		L	2591.90	
MS	Peaks	-	920.05	1218.29	1346.67	1516.69	1593.72	1719.91	1953,12	(327,75	157070	670765	714717	(183.48	1216.39	1354.03	1507.81	1557.75	2740,34	2591.60	
Anc No			P97351 (Swite- Prof)							67658 (HCBI)	1			P12750 (Swiss- Prof)					•		
	S Trans		Mouse							Human				Human							
	Danilla		7 peaks match	253			WEET-			Trypsia 4 peaks metch	Hic		-	Trypsin 6 peaks match with scosume	3						
	5360		Thypsin							Trypsie				Trypsin							
	pods		Φ.							2				=							

Figure 20. Quail Chorioallantoic Membrane (CAM) Angiogenesis Assay

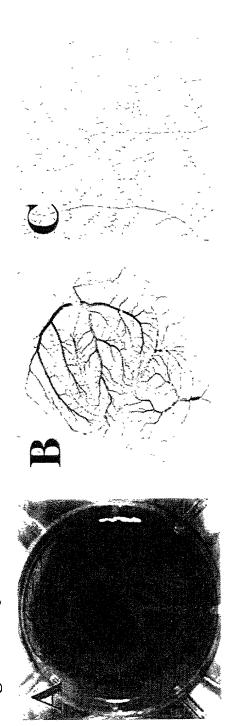


Figure 21. Black and white images of CAM vasculature after growth factor treatment

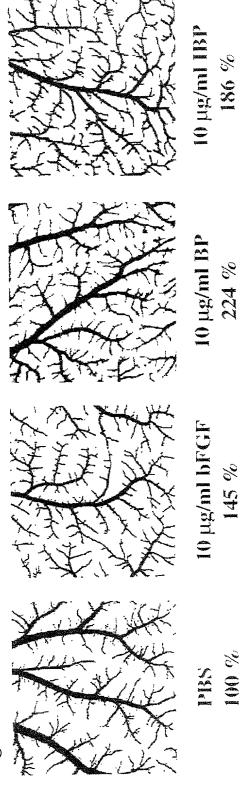
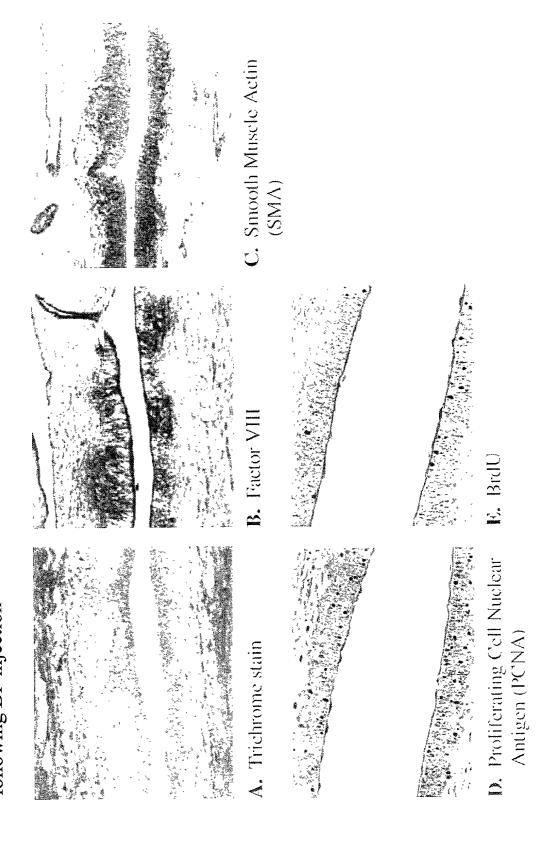


Figure 22. Histological sections of blood vessels formed in canine myocardium 2 weeks following BP injection



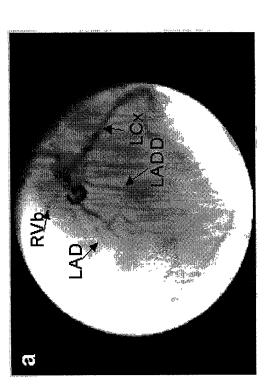


Fig. 23

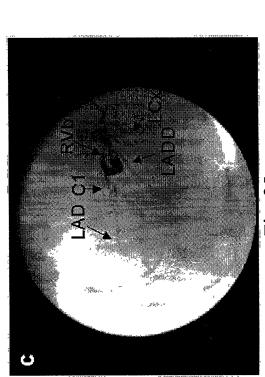


Fig. 25

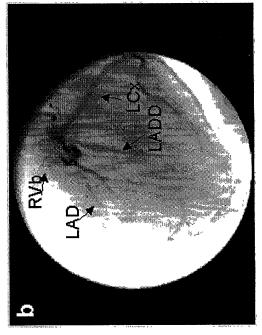


Fig. 24

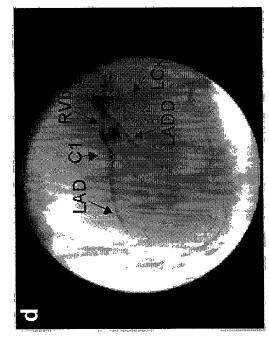


Fig. 26